

Dominance of *Capsicum minutiflorum* (Solanaceae) pollen in stingless bee hives: An insight into protein composition and foraging behavior by four Meliponini species of the Bolivian-Tucumano forest

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Abstract

Stingless bees (Apidae: Meliponini) primarily feed on nectar and pollen from a wide diversity of flowering plants. By doing so they pollinate these flowers thus contributing to biodiversity and ecosystem stability. The pollen they collect provides essential nutrients for brood rearing and colony growth. This study aimed to characterize the floral resources available to stingless bees in a Tucumano-Boliviano Forest, including their pollen protein content and, through construction of an interaction network and preference analysis, understand their foraging behavior. Only 8 out of 25 pollen types sampled within the study site around the meliponaries were collected by the bees. Pollen pots also contained many types of pollen not from to plant species in the study area. Pollen from *Capsicum minutiflorum* (Solanaceae) was dominant in almost every hive (up to 98.7% of pollen composition). Additionally, protein content of *Capsicum minutiflorum* pollen (67% w/w) was the highest of all species present at the study site and explained almost 100% of the protein content in the hives of *Tetragonisca angustula*, and *Scaptotrigona depilis*, and almost 80% and 75% of the protein content in those of *Scaptotrigona polysticta* and *Melipona rufiventris*, respectively. These results suggest that stingless bees preferentially collect pollen with higher protein content.

Keywords

Bee nutrition, *Melipona*, plant–pollinator interactions, pollen preferences, *Scaptotrigona*, *Tetragonisca*

Introduction

Bees are considered among the most effective pollinators, and they play a fundamental role in the maintenance of biodiversity and stability of ecosystems. This is especially true in the tropics, where due to the high plant diversity, and consequent low density of the different plant species, plants are more dependent on their pollinators (Absy et al. 2018; Klein et al. 2018; Rodger et al. 2021). Thus, in the tropics nearly 94% of all flowering species are animal pollinated, and most of these animals are bees (Ollerton et al. 2011). In most regions of the neotropics, stingless bees (Apidae: Meliponini) are very abundant and very diverse, with around 400 species of the 550 species described worldwide being found in such habitats (Barbiéri et al. 2022). Stingless bees are eusocial insects that live in perennial colonies with 3,000 to 100,000 individuals (Michener 2007, 2013). In Bolivia, more than 100 stingless bee species have been described (Townsend et al. 2021), and as in many other countries of Latin America, they hold great cultural value for many indigenous and rural populations. (Souza et al. 2013; Reyes-Gonzalez et al. 2014). Stingless bee breeding, formerly called meliponiculture, is primarily practiced for their honey and for other useful resources such as cerumen, propolis, and pollen, which have various religious, medical, nutritional and economic uses (Vit et al. 2013, 2018; Salatino 2019; Adler et al. 2023).

Stingless bees feed on nectar as their primary carbohydrate source and pollen as main protein source, although pollen also provides amino acids, lipids, carbohydrates, sterols, and various micronutrients; thus, pollen constitutes a nutritionally diverse and highly valuable reward (Roulston and Cane 2000; Vossler 2015). Pollen is also important for brood rearing and development, since it is the main ingredient of larval food, and hence its availability directly influences brood rearing and the growth of the colony (Roubik 1982; Abrol 2012). Thus, to maintain a healthy colony, worker bees must be efficient in collecting floral pollen that satisfies its nutritional requirements. In that sense, a preference for high-quality proteins or a high protein content in the diet has been observed in species that are part of the same subfamily (Apinae), to which stingless bees belong. For example, in honey bees (*Apis mellifera*), depending on the colony's needs, foragers will search for pollen with higher protein content, although protein-lipid ratio and amino acid content plays also a role in their preference (Ghosh et al 2020). Bumble bees (*Bombus*) fed artificially with high protein diets, enhance their colony growth (Génissel et al 2002) and in the wild they prefer flower species with higher protein levels that those chosen by honey bees (Leonhardt and Blüthgen 2011). In Stingless bees colonies, higher protein content in the larval food can have a positive effect on body size of worker bees (Quezada-Euán et al. 2011), but if they forage preferentially on pollen with higher protein, has not yet been studied. To avoid competition stingless bees have developed different strategies to recruit their nestmates and tell them where the food sources are located. Depending on the species some have strong recruitment

strategies, like most *Scaptotrigona* species, and some weak or no recruitment strategy, like *Melipona* or *Tetragonisca* species (Ramalho 1989; Michener 2013; Grüter 2020). Although other bee tribes belonging to the “corbiculate” clade, such as Bombini (Vaudo et al. 2016; Ruedenauer et al. 2020) and Apini (Pernal et al. 2001; Cook et al. 2003; Gosh et al. 2020) have distinguishable preferences and foraging behavior towards specific nutrients in pollen to fulfill their nutrient requirement, these preferences have been poorly studied in species from the Meliponini tribe (Grüter 2020).

As meliponiculture in Bolivia continues to grow as a socio-economic activity, particularly for vulnerable populations, it becomes important to conduct in-depth studies of the behavior and requirements of these bees (Adler et al. 2023). A comprehensive understanding of the system will facilitate improved management practices and lead to enhanced productivity and conservation efforts both, of the bees and the ecosystem. This is of particular importance in the Tucumano-Boliviano forest of Chuquisaca, where large-scale meliponiculture is performed by women from different communities. The present study, performed in the protected area of Serranías del Iñaño, a region that remains largely understudied, aims to characterize the floral resources available within the foraging range of stingless bees from three meliponaries. Furthermore, we seek to quantify the protein content of various pollen species to enquire about preferential foraging behaviors, thus aiming to provide insights that can lead to sustainable meliponiculture practices and ensure the well-being of both the bees and the communities that rely on them.

Methods

Study area

The Bolivian-Tucumano forest is characterized by a mesotropical subhumid pluviseasonal bioclimate, with an average annual rainfall of 915 mm and an average temperature of 25 °C. The upper forest occupies an altitudinal range between 1100 and 1800 meters above sea level. The most common plant species in the area are “cedro” (*Cedrela lilloi*), “tipa” (*Tipuana tipu*), “morado” (*Machaerium scleroxylon*), “palo barroso” (*Blepharocalyx salicifolius*), “mato” or “sahuinto” (*Myrcianthes pseudomato*), “laurel” (*Cinnamomum porphyria*), “roble” (*Amburana cearensis*), “nogal” (*Juglans australis*), among others (Navarro and Ferreira 2011a, b; SERNAP 2013). Our study was carried out within this distinct ecoregion, nearby the community Ticucha in the Chuquisaca department, Bolivia (Fig. 1). The survey area contained three different melliponaries, located at a distance of about 250 m from each other: 1) Meliponary 1 “Mel-1” (19°37'30.44"S, 63°48'21.53"W), where samples of *Tetragonisca angustula* were taken, 2) Meliponary 2 “Mel-2” (19°37'37.85"S, 63°48'32.85"W), where samples of *Scaptotrigona depilis* and *Scaptotrigona polysticta* were taken and 3) Meliponary 3 “Mel-3” (19°37'47.51"S, 63°48'34.35"W), where samples of *Tetragonisca angustula* and *Melipona rufiventris* were taken. No anthropic activity is currently carried out near the melliponaries, so they were surrounded by forest and are near a branch of the Ñancahuasu river (Fig. 1).

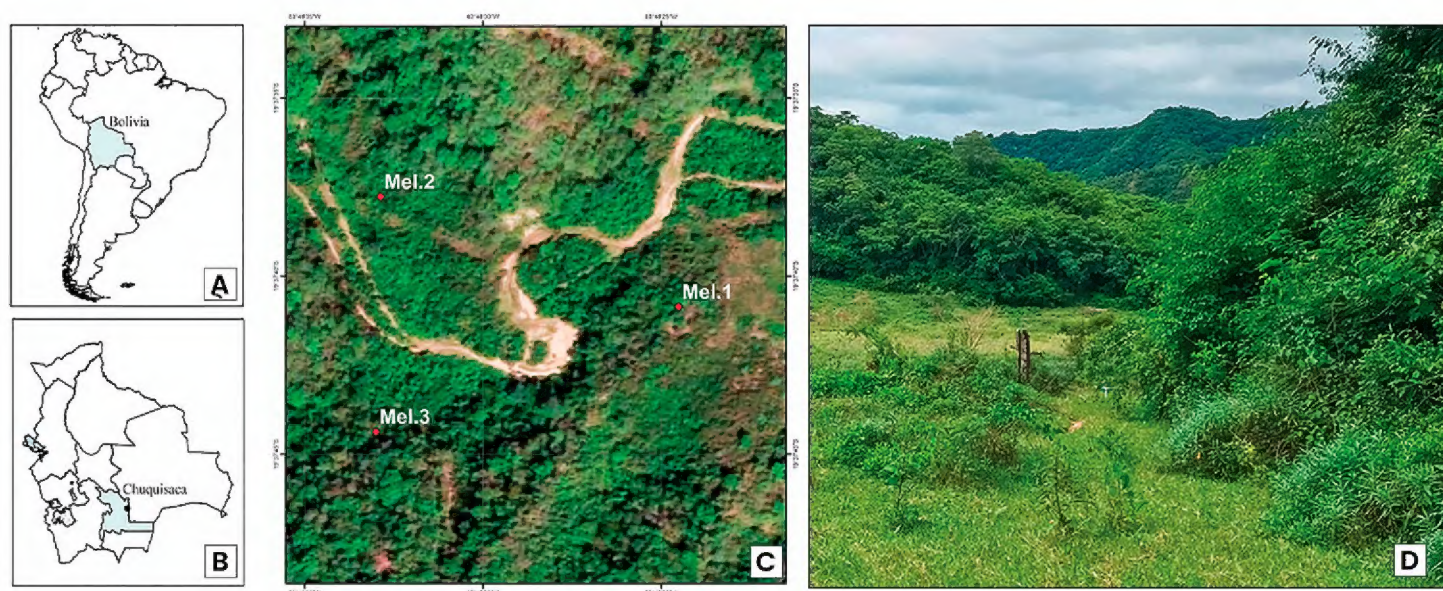


Figure 1. **A** map of South America, locating in green Bolivia **B** map of Bolivia, locating in green the department of Chuquisaca. The black dot point the study area **C** satellite image of the study area within the department of Chuquisaca. The red dots point the location of the meliponaries **D** vegetation in the study area.

Botanical and floral pollen sampling

Floral and pollen sampling was conducted at the end of the rainy season (March) in 2022, to determine the pollen offer available for the bees during the study period. To establish the sampling area surrounding the meliponaries, the estimated flight range of the studied bee species (*Tetragonisca angustula*, *Scaptotrigona depilis*, *S. polysticta* and *Melipona rufiventris*) was considered, resulting in a sampling plot of 800 × 800 m around each meliponary (Nogueira-Neto 1997; Araújo et al. 2004; Greenleaf et al. 2007; De Oliveira Alves et al. 2018; Urquiza et al. 2022). These 800 × 800 m plots were divided into 64 sub-plots (100 × 100 m, Fig. 2A). In each of the sub-plots, eight 20 m-transects (Fig. 2B) were established along which all flowering individuals were counted and their growth form (herb, shrub or tree) characterized. Samples from each individual were collected and pressed for subsequent taxonomic identification. Several flowers per species were collected to obtain enough pollen (2 g) for protein content analysis and for construction of a pollen reference collection of the area (5 flowers were used for quantification and identification of pollen). Also, the flowers from herbs were sampled from four quadrants (1 × 1 m; Fig. 2B) and every individual within the quadrant was counted.

For the identification of the botanical specimens, the family was first determined by comparing them with the collections of the Herbarium of Southern Bolivia (HSB). Additionally, a comparative analysis was performed by comparing the specimens with the descriptions available on the World Flora Online platform (www.worldfloraonline.org) and the Catalog of the Vascular Plants of Bolivia (Jørgensen et al 2014). Subsequently, specialized literature was consulted, including the work of Davenport (2004), Knapp (2010), Jiménez et al (2011), and Barboza et al. (2022) to confirm the genus and species, when possible.

In order to estimate pollen demand, pollen was extracted directly from 3 pollen pots of each hive and mixed together. Pollen was extracted from 8 hives of

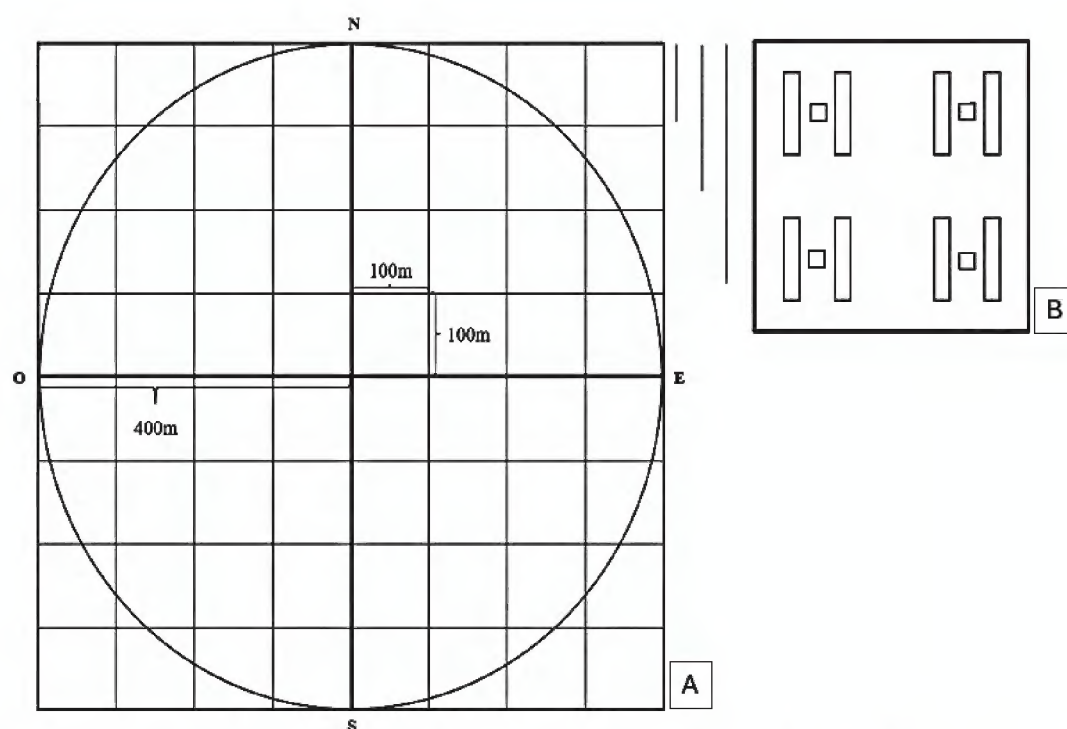


Figure 2. **A** floral and pollen sampling design. The main plot (**A**) with the meliponary in the center is divided into 64 subplots. Each subplot has eight transects and 4 quadrants where flowers were collected (**B**).

Tetragonisca angustula, locally known as “señorita”. Of these 8 hives, 5 were in Meliponary 1 (Mel-1) and 3 in Meliponary 3 (Mel-3). Samples from 2 hives of *Melipona rufigiventris*, locally known as “Erereú”, were collected from Mel-3. Finally, samples from 5 hives of *Scaptotrigona depilis* and 5 hives of *Scaptotrigona polysticta*, both locally known as “Negros”, were collected from Meliponary 2 (Mel-2). All pollen samples were kept in sterile vials with filter paper at a temperature of 4 °C.

The collected plant material was identified by specialists using taxonomical keys. Voucher specimens served afterwards as a reference collection for the pollen reference collection.

The pollen collected from the flowers in the study area (5 flowers of each species) and the pollen collected from the beehive pots were subjected to an acetolysis process (Erdtman 1969) in which the cellular content of pollen grains was destroyed leaving only the outer wall or exine, clean and colored, which served to identify the pollen grains. Identification was carried out to the highest possible taxonomic category using the reference pollen reference collections and available literature.

Acetolyzed pollen was observed through an optical microscope (AmScope MU1000) at 400 × magnification. It was photographed using a Boeco digital camera (B-CAM10), and measurements of the lengths of the polar and equatorial sides were recorded. To assess the volume of pollen grains, they were assigned to distinct geometric shapes following Vossler (2015): sphere, ellipsoid, and triangular-based prism. Pollen grain size was ascribed to five categories as previously done by Hesse et al. (2009) and Vossler (2015): very small (< 10 µm), small (10–25 µm), medium (26–50 µm), large (51–100 µm) and very large (> 100 µm).

To quantify the different pollen types in the flowers and the pots, a Neubauer chamber was used into which 15 µl of the final acetolysis solution was deposited and

pollen grains counted. This procedure was repeated three times, and the three counts were averaged. After all pollen types were quantified the percentage of occurrence of each pollen type was determined and the Neubauer formula was applied to calculate pollen density in the flowers (Dafni et al. 2005).

Protein quantification analyses in the pollen

Protein analyses were performed following the Bradford protocol (Roulston et al. 2000). First, a calibration curve was created using known concentrations of bovine serum albumin (BSA). Next, the dried pollen samples (2 g) were pulverized with steel balls and prepared for absorbance reading at 595 nm in a Boeco S-220 spectrophotometer. Each sample was measured in triplicate and results expressed as percentage.

Analysis

The Sorensen's Similarity Index (SSI) was calculated to evaluate the similarity of the pollen species in the pots at the intra- and inter-specific levels (Sørensen 1957).

Spearman's rank correlation was used to test for an association between the volume, relative abundance and protein content of pollen.

To construct an interaction network between pollinators and plants visited, a sampling method was chosen which was based on the pollen volume found in the pollen pots from the hives. The use of stored pollen has several advantages over observing visitation rate. Firstly, it results from the temporal accumulation of interactions between bees and plants over a moderately long period of time. Secondly, only the pollen actually collected is stored, which avoids considering casual visits to flowers that are not a regular part of the bees' preferences. Thirdly, it allows for the recording of interactions with plants that may have low abundance in the study system and would not have been detected otherwise (Bosch et al. 2009).

To better visualize this network, an interaction matrix was constructed between the four bee species and the plant species (or pollen morphotypes in the case of unidentified or unobserved plant species in the area). The intensity of the interaction was defined as the proportion of pollen volume from a particular plant species found in the pots. Volume was used instead of number of pollen grains because the presence of numerous small pollen grains in the sample could bias the result in favor of plant species with such pollen type. Several metrics were calculated of the interaction network using the network level functions of the bipartite package of the free software R version 4.2.1 (2023). Thus, the network's structure was described using metrics of specialization (H2'), connectance (C), modularity (Q), and nestedness (NODF). Additionally, the measure of specialization of each of the bee species was calculated using the Shannon diversity index, as an independent measure of the network structure.

For each bee species, the percentage of the pollen volume in the beehive pots originating from each plant species was calculated. Only plants contributing more than 1% of the total volume were considered. Similarly, percentage contribution of each plant species to this content estimated.

The protein content of a pot (CP) was calculated as follows:

$$CP = \sum prot_i \times voli \times grains_i$$

where: $prot_i$ represents the protein contribution of plant species i , vol is the volume of its pollen grain and $grains$ is the number of grains of that species found in the pots.

Data resource

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Results

A total of 25 plant species categorized into 17 families, were at the flowering stage in the sampling area defined. Most of the observed species were herbs (16 species), and only 4 species of lianas, 4 of trees and one bush showed flowers during the survey period. The dominant plant species at the flowering stage was identified as the liana *Heteropterys umbellata* (Malphiaceae), with a relative abundance of 13.64%, followed by the herb *Asclepia curassavica* (Apocynaceae) (10.91%) (Table 1).

Pollen density, defined as the number of pollen grains per unit volume and calculated using the Neubauer formula, varied significantly among the studied plant species. The highest pollen density was recorded in *Piper elongatum*, with 1392045 pollen grains per mm^3 . *Ludwigia peruviana* followed with less than 1/10 of pollen density (11267), and the flowers with the lowest pollen density were *Oxypetalum* sp. with just 12 pollen grains per mm^3 , and *Asclepia curassavica* with 22 pollen grains per mm^3 (Table 1). Unlike volume-based measurements, this approach highlights actual differences in pollen grain abundance, which is particularly relevant for evaluating pollen availability for pollinators.

In terms of cytoplasmic volume, the species with the grates cytoplasmic volume was *Sida anrgetina* (Malvaceae), with 1334016 μm^3 , followed by *Sida* sp. (Malvaceae) with 782698 μm^3 . On the other hand, the species with the smallest volume was *Piper enlongatum* (Piperaceae) with 699 μm^3 , followed by *Oxypetalum* sp. (Apocynaceae) with 1921 μm^3 . No significant correlation was found between pollen quantity and cytoplasmic volume ($p = 0.1911$, $\rho = -0.2704$), suggesting that pollen grain abundance does not necessarily correspond to pollen grain size.

The protein content from the collected flower species varied from 67% in *Capsicum minutiflorum*, to 20% in *Piper elongatum* (Table 1). No significant correlation was found between protein content and pollen quantity ($p = 0.495$, $\rho = 0.1463$) or pollen volume ($p = 0.5248$, $\rho = -0.1365$).

In total, in all pot-pollen samples, 23 pollen types were identified: 14 in the pollen types from *M. rufiventris*, 13 in *S. polysticta*, 10 in *S. depilis* and 5 in *T. angustula* (Table 2).

Table 1. Family and species of the floral species at the flowering stage in the study site available by the end of the rainy season in the Boliviano-Tucumano Forest of Chuquisaca, Bolivia. The four largest values of each data column are highlighted in bold type.

Family	Specie	Habitus	Relative abundance (%)	Pollen density (grains/mm3)	Proteic content (%w/w)	Pollen Grain Volume (µm3)	Pollen size category
Acanthaceae	<i>Justicia ramulosa</i>	Herb	5.45	45	0.29	106453	Large
Apocynaceae	<i>Asclepia curassavica</i>	Herb	10.91	22	0.29	4831	Small
Apocynaceae	<i>Oxypetalum</i> sp.	Liana	5.45	12	0.52	1921	Small
Asteraceae	<i>Hymenostephium jebile</i>	Herb	2.73	200	0.31	16914	medium
Asteraceae	<i>Pectis</i> sp.	Herb	2.73	988	0.35	6143	Small
Asteraceae	<i>Heterosperma</i> sp.	Herb	0.91	300	0.43	4280	Small
Commelinaceae	<i>Commelina</i> sp.	Herb	2.73	185	0.26	30178	medium
Convolvulaceae	Convolvulaceae sp.	Liana	4.55	245	0.54	37288	medium
Euphorbiaceae	<i>Croton</i> sp.	Herb	1.82	100	0.48	130240	Large
Fabaceae	<i>Inga saltensis</i>	Tree	4.55	133	0.50	529117	very Large
Fabaceae	<i>Desmodium</i> sp.	Herb	1.82	100	0.43	119932	Large
Fabaceae	<i>Inga adenophylla</i>	Tree	0.91	167		9350	medium
Lamiaceae	<i>Salvia</i> sp.	Herb	0.91	278	0.44	7710	Small
Malpighiaceae	<i>Heteropterys umbellata</i>	Liana	13.64	267	0.47	41167	medium
Malvaceae	<i>Sida argentina</i>	Herb	0.91	312	0.29	1334016	very Large
Malvaceae	<i>Sida</i> sp.	Herb	0.91	155	0.40	782698	very Large
Onagraceae	<i>Ludwigia peruviana</i>	Herb	5.45	11267	0.40	66007	Large
Piperaceae	<i>Piper elongatum</i>	Herb	4.55	1392045	0.20	699	Small
Plumbaginaceae	<i>Plumbago</i> sp.	Herb	1.82	67	0.34	189948	Large
Rubiaceae	<i>Hamelia patens</i>	Tree	4.55	5300	0.52	9729	medium
Rubiaceae	<i>Richardia</i> sp.	Herb	4.55	3112	0.52	8074	Small
Sapindaceae	<i>Serjania</i> sp.	Liana	6.36	112	0.47	5423	Small
Solanaceae	<i>Capsicum minutiflorum</i>	Bush	6.36	533	0.67	8330	Small
Solanaceae	<i>Solanum riparium</i>	Tree	4.55	433	0.47	2719	Small
Talinaceae	<i>Talinum</i> sp.	Herb	0.91	78	0.24	140109	Large

A very eye-catching result was the dominance of one particular pollen species in almost every hive studied (Fig. 3). Pollen from *Capsicum minutiflorum* (Solanaceae) was always dominant in every hive, except in hive 1 (H1) from *S. polysticta*, which showed a more balanced pollen distribution. In this case, *Capsicum minutiflorum* represented in this hive 17% of the total pollen, while *Solanum riparium* and Proteaceae sp1. represented 36.3% and 32.5%, respectively.

The average for every bee species, pollen composition between the different species, varied from very different to similar, with a Sorensen index between 26.7% to 69.6% similarity. The Sørensen Similarity Index (SSI) calculated for the percentage of the different pollen types found in the pollen samples from every beehive from the four bee species, demonstrated that between hives of the same species, there was almost always a great similarity (over 50%). The only exception was *S. polysticta* where some hives showed low similarity indexes (Table 3).

Based on pollen species composition found in the pot pollen for every bee species, the calculated network exhibited an intermediate level of complementary specialization with $H' = 0.516$, a high connectance with $C = 41.667\%$, an intermediate level of nestedness with a NODF value of 49.603, and nearly no modularity with $Q = 0.163$.

Table 2. Pollen types identified and pollen species composition. Percentage (%) of pollen volume in the pot-pollen from the four bee species studied. Msp stands for the unidentified morphospecies. Species that were also collected in the study site are highlighted in bold type.

Family – Genus	<i>Melipona rufiventris</i> (%)	<i>Scaptotrigona depilis</i> (%)	<i>Scaptotrigona polysticta</i> (%)	<i>Tetragonisca angustula</i> (%)
<i>Alternanthera</i> sp.	0	0.2	0	0
Amarantaceae sp.	0	0	0	0
<i>Baccharis</i> sp.	0	0	0	0
<i>Capsicum minutiflorum</i>	66.6	96	71.9	98.8
Convolvulaceae sp.	0	0	0	0
Croton sp.	0	0.7	0.2	0
<i>Desmodium</i> sp.	0	0	0	0
<i>Hamelia patens</i>	0	0	0	0
<i>Heteropterys umbellata</i>	0	0	0	0
Heterosperma sp.	0	0	0.2	0
<i>Inga adenophylla</i>	0.1	0	0	0
<i>Inga saltensis</i>	0.1	0	0	0
Msp 1	0	0	0	0
Msp 2	0	0	0.3	0
Msp 3	0	0	0.4	1.2
Myrtaceae sp. 1	0	0	0	0
Myrtaceae sp. 2	0	0	1.3	0
Plumbago sp.	10.1	0	0	0
Poaceae sp.	0	0	0	0
Primulaceae sp. 1	0.1	0	0.1	0
Primulaceae sp. 2	0	0	0	0
Proteaceae sp. 1	10.7	2.6	23.2	0
Proteaceae sp. 2	11.5	0	0	0
Sapindaceae sp.	0.5	0.4	0.8	0
<i>Solanum riparium</i>	0	0	1.6	0
<i>Zuccagnia punctata</i>	0.3	0	0	0

Interactions were predominantly driven by the interaction between the four bee species and *Capsicum minutiflorum* (Fig. 4). However, interaction did not occur equally across the four bee species. In *S. depilis* and *T. angustula*, the pollen of *C. minutiflorum* accounted for 96% and 98.8%, respectively. In contrast, the value of the interaction was 66.6% for *M. rufiventris* and 71.9% for *S. polysticta*.

Regarding the proportion of the protein content in the stored pots that can be attributed to each plant species, *Capsicum minutiflorum* explained almost 100% of the protein content in *T. angustula* and *S. depilis*. However, almost 20% of the protein content in the reserves of *S. polysticta* could be attributed to Proteaceae sp.1, and almost 25% of the protein content in the reserves of *M. rufiventris* could be attributed to *Plumbago* sp., Proteaceae sp.1 and Proteaceae sp.2 pollen grains (Fig. 5).

Discussion

According to the width of the taxonomic spectrum of plant species used as pollen sources by bees, they are classified as oligolectic if they forage on plant species belonging to one or several closely related genera, and as polylectic if they obtain pollen

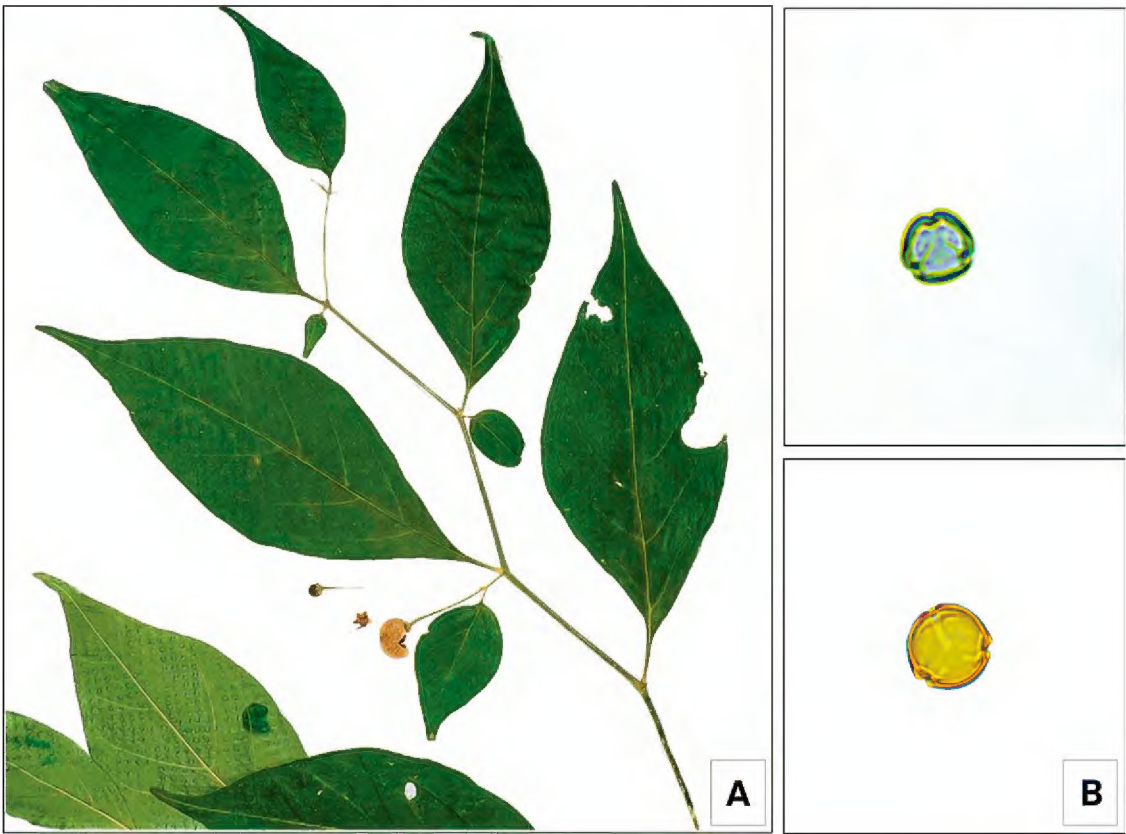


Figure 3. *Capsicum minutiflorum* **A** dried specimen **B** acetolyzed pollen

Table 3. Sorensen similarity index between bee hives of the same bee species. SSI was calculated between hives (H1 to H5) of the same species in each meliponary.

Hives	Sorensen similarity Index				
	Meliponary 1	Meliponary 2		Meliponary 3	
	<i>T. angustula</i>	<i>S. depilis</i>	<i>S. polysticta</i>	<i>M. rufiventris</i>	<i>T. angustula</i>
H 1 - H 2	80.00	80.00	61.54	92.32	0.99
H 1 - H 3	66.67	80.00	42.86	–	1.00
H 1 - H 4	66.67	75.00	75.00	–	0.99
H 1 - H 5	100.00	71.43	63.16	–	–
H 2 - H 3	57.14	100.00	66.67	–	–
H 2 - H 4	57.14	82.35	72.73	–	–
H 2 - H 5	80.00	93.33	28.57	–	–
H 3 - H 4	100.00	82.35	66.67	–	–
H 3 - H 5	66.67	93.33	53.33	–	–
H 4 - H 5	66.67	87.50	58.82	–	–

from plants from two or more families (Roulston et al. 2000; Murray et al. 2009). However, it has been proposed that even in polylectic species there is some degree of selectivity and bees do not collect pollen from all plant species available (Nicholls and Hempel de Ibarra 2017). Some stingless bees have been considered polylectic (Oliveira et al. 2009; Rech and Absy 2011; Vossler 2012), while others are more selective in terms of pollen collection (Oliveira et al. 2009; Rech and Absy 2011). For example, Aleixo et al. (2017), and later Vossler (2018) suggests that some known polylectic stingless bee species may collect pollen from only one or a few plant species, even when the floral offer is large; moreover, due to a flexible foraging behavior, false conclusions may be drawn on the category of specialization if data from a single nest is gathered.

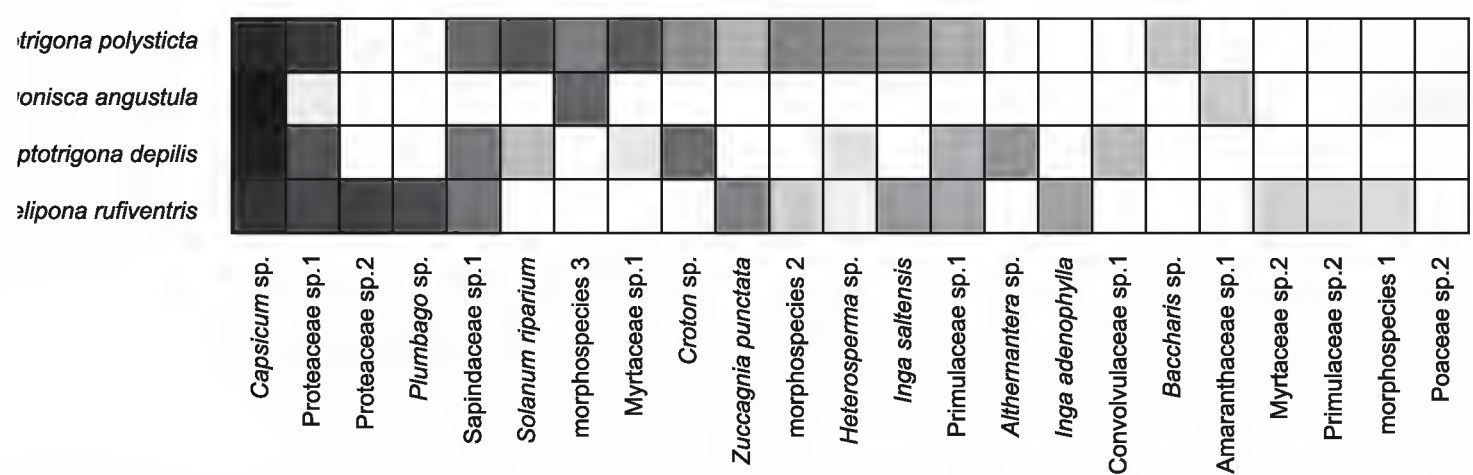


Figure 4. Bipartite quantitative interaction matrix based on the pot pollen (green) found in the hives for every bee species (red). Interaction intensity is represented by gray shade.

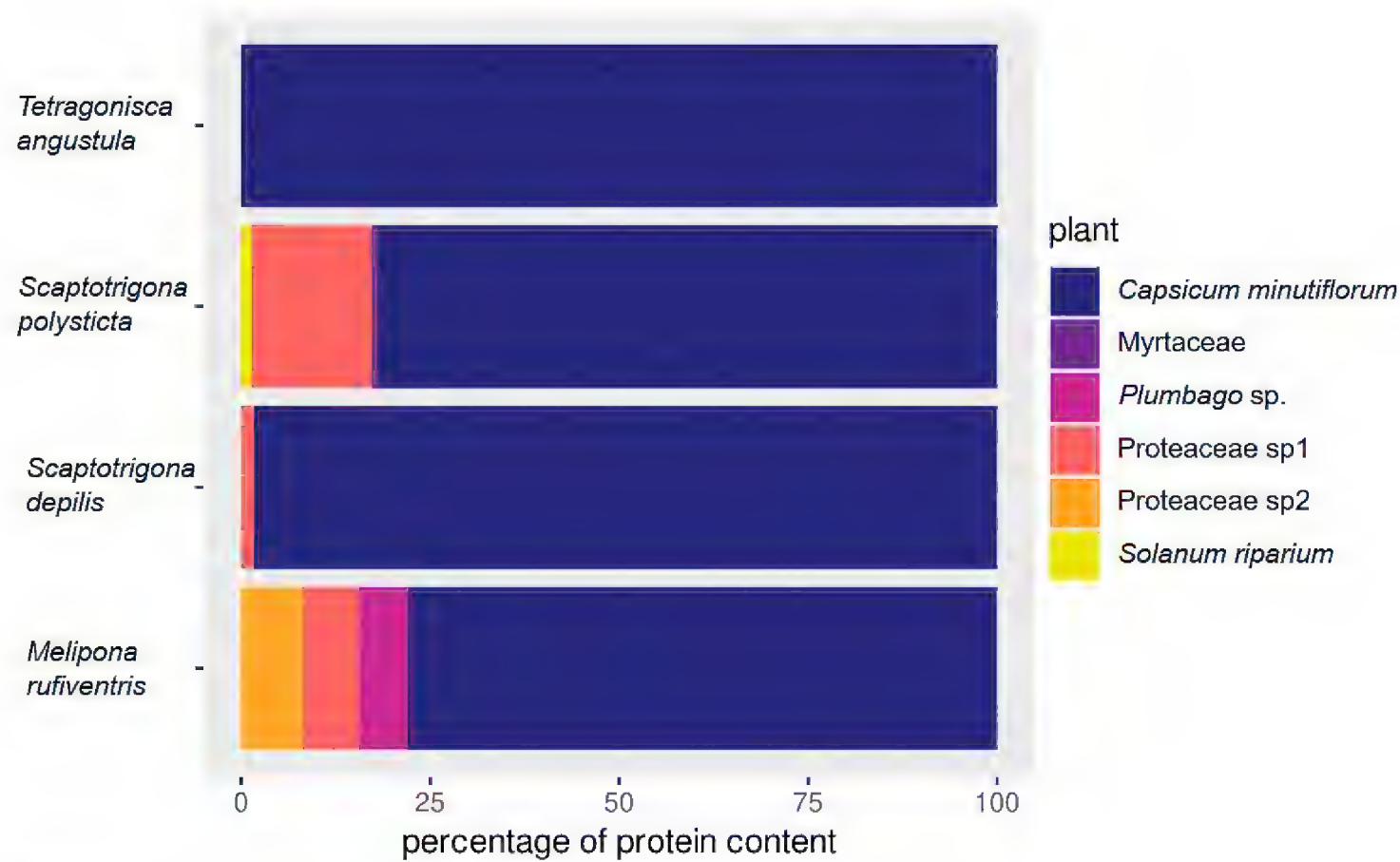


Figure 5. Percentage of protein content collected by each bee species.

The present results show that the species studied are polylectic but nevertheless exhibit pollen selectivity. This conclusion is based on three facts: a) the diversity of pollen types that were collected which belong to more than one family; 9 in *M. rufiventris* (Asteraceae, Fabaceae, Myrtaceae, Plumbaginaceae, Primulaceae, Proteaceae, Sapindaceae, Solanaceae, and one unidentified family), 10 in *S. polysticta* (Solanaceae, Proteaceae, Sapindaceae, Myrtaceae, Asteraceae, Primulaceae, Fabaceae, Euphorbiaceae, and two unidentified families), 9 in *S. depilis* (Solanaceae, Proteaceae, Sapindaceae, Myrtaceae, Amaranthaceae, Asteraceae, Primulaceae, Euphorbiaceae, and Convolvulaceae) and 5 in *T. angustula* (Solanaceae, Proteaceae, Poaceae, Amarantaceae and one unidentified family, b) only 8 out of 25 pollen types identified around the meliponaries

within the study site were collected by the bees studied; 6 in *M. rufiventris* (*Capsicum minutiflorum*, *Plumbago* sp., Sapindaceae sp., *Heterosperma* sp., *Inga adenophylla*, *I. saltensis*), 5 in *S. polysticta* (*C. minutiflorum*, Sapindaceae sp., *Heterosperma* sp., *Croton* sp., *I. saltensis*), 5 in *S. depilis* (*C. minutiflorum*, Sapindaceae sp., *Heterosperma* sp., *Croton* sp., Convolvulaceae sp.), and 1 in *T. angustula* (*C. minutiflorum*). And c) the pollen collected by the four bee species belong mostly to a single plant genus; *Capsicum*.

Bees consume nectar for their own nourishment and typically collect pollen (plus nectar) as food for their offspring, transporting it to the nest via body hairs, scopae or using corbiculae (in the case of eusocial corbiculate bees) (Michener 2007; Nicholls and Hempel de Ibarra 2017). Pollen protein content is crucial for reproduction, growth and longevity of bees, and there is a correlation between performance of bees and the amount of protein consumed (Cane and Buchmann 2000; Cook et al. 2003; Weiner et al. 2010; Quezada-Euán et al. 2011). While earlier studies argued that some bees primarily collect pollen based on floral availability rather than protein content, such as was reported for *Apis mellifera* (O’Neal and Waller 1984), newer evidence suggests that nutritional quality does influence their foraging decisions, particularly when resources are scarce or colony needs are high. For example, some studies carried out with *Bombus* and *Apis* species, which are polylectic and eusocial bees (similarly to the four bee species herein studied), have reported that pollen collected for the provisioning of larvae shows high protein concentration (Robertson et al. 1999; Cook et al. 2003; Ghosh et al. 2020). One study carried out with *Apis mellifera* reported high protein content in pollen load samples collected directly from hives and demonstrated that pollen types most frequently found in samples have also the highest protein content (Sá-Otero et al. 2009). Nevertheless, more recent studies have expanded on this, showing that protein-lipid ratios also play a critical role in bee foraging preferences and colony health (Vaudo et al. 2020). These findings align with observations reported in this study; Stingless bees showed a preference for high-protein pollen types, such as *C. minutiflorum*, over other abundant but lower-protein species. Additionally, studies on Stingless bees in various regions, including those by Vossler (2018), have emphasized the strong linkage between pollen preferences and nutritional value. Similar patterns have been observed in Amazonian *Melipona* species, where Fabaceae and Myrtaceae are frequently dominant in pollen stored inside the meliponaries (Ferreira and Absy 2015). Moreover, studies on *Tetragonisca angustula*, such as those by Braga et al. (2012) and De Novais et al. (2014), reveal significant variation in pollen use across different ecosystems, further supporting the idea that Stingless bees adapt their foraging strategies to local floral availability and nutritional demands.

Thus, differences in protein content may influence bees’ foraging decisions (Keller et al. 2005), which require a sensorial capacity allowing them to select the type of pollen they collect. Bees sample pollen grains with their mouthparts and antennae during pollen manipulation, a behavior that has been linked to the detection of nutritional quality (Nicholls & Hempel de Ibarra 2017; Ruedenauer et al. 2020). However, preference for pollen is also affected by plant proximity to the hive (Sá-Otero et al. 2009), competition (Ferreira and Absy 2015) and prior foraging experience, factors that may lead to the selection of a familiar pollen type with lower protein concentration

(Nicholls & Hempel de Ibarra 2017). Interestingly, Urquizo et al. (2022) demonstrated that *Tetragonisca angustula* often selects Solanaceae pollen, underscoring the importance of nutrient-rich families in shaping foraging preferences. However, the pronounced reliance on the single genus *Capsicum* reported in this study contrasts with broader diets, such as those reported for *T. angustula* by Saravia-Nava et al. (2018). The contrast between those results and the ones reported here may be explained by the influence of both regional and seasonal factors on foraging behavior.

The present results show that bees are indeed able to sample pollen differentially since, in spite of the high abundance and diversity of plant species surrounding the meliponaries they collected few pollen types and showed strong preference for one (i.e., *Capsicum minutiflorum*) with the highest protein content. Moreover, other species with relatively high protein content and abundance (e.g. *Oxypetalum* sp., *Richardia* sp. and *Hamelia patens*) were collected in much smaller proportions than *C. minutiflorum*, and three plant species (*Asclepia curassavica*, *Heteropterys umbellate* and *Serjania* sp.) around the hives which had the same or more relative abundance than *C. minutiflorum* (*Asclepia curassavica*, *Heteropterys umbellate* and *Serjania* sp.) and lower protein content than *C. minutiflorum* were not found in pollen pots of the hives.

The foraging preferences of Stingless bees presented in this study align with findings obtained in Stingless bees from Brazil, where families like Fabaceae and Myrtaceae frequently dominate the diets (Vossler 2021). However, the dominant use of *Capsicum* pollen, as reported in this study, is a novel observation because *Capsicum* has rarely been reported in pollen stores from Brazilian or Amazonian studies. Guimarães et al. (2021) observed that *Melipona seminigra* shows strong preferences for Fabaceae and Euphorbiaceae in disturbed and natural environments, suggesting that floral availability and nutritional needs drive foraging choices. Similarly, Braga et al. (2012) found that *T. angustula* relies on diverse pollen sources depending on seasonal floral availability. Nevertheless, none of both studies documented *Capsicum* dominance. This discrepancy underscores the importance of conducting regional studies to identify how local floral resources influence foraging behavior.

Solitary bees construct a pollen mass inside of each cell where immature develops, as results of several foraging flights (Michener 2007). These pollen masses constitute the food that larvae consume. Thus, longer foraging flights affect female brood productivity and offspring survival; the number of brood cells provisioned per unit time decrease substantially with increasing foraging distance, so energy and time investments resulting from longer foraging distances reduce pollen quantity used for provisioning their offspring (Peterson and Roitberg 2006; Zurbuchen et al. 2010). By the contrary, in *Apis mellifera*, a eusocial species that builds combs where colonies develop, the amount of pollen is collected in relation to the colony's need, which depend on the number of larvae and adult bees that constitute the colony (Ghosh et al. 2020). *M. rufiventris*, *S. polysticta*, *S. depilis* and *T. angustula* are eusocial species that belong to the "corbiculate" group. That is, they have a specialized structure that serves for transport of a large amount of pollen (Michener 1999). We think that these species have similar colony's needs based on the fact that they exhibit similar pattern of resource

use: preference for the same type of pollen in all four species, very high amounts of that pollen in all four species, and high similarity in the composition of pollen between hives of the same species, in three of the four species.

Quantity and size of collected pollen may also influence foraging decisions. Thus, bees may collect higher quantities of nutritionally poor pollen to compensate for its low quality (Wcislo and Cane 1996). This was not the case for *M. rufiventris*, *S. polysticta*, *S. depilis* and *T. angustula*, according with data herein reported. Thus, only three pollen types categorized as “large” or “very large” (*Croton* sp., *Inga saltensis* and *Plumbago* sp.) were collected by two of the four bee species, and they were collected at very low proportions. Since all species studied are polylectic, they appear to choose the pollen type that offers the highest protein content, independent of its grain size or relative abundance in the home range of bees. Thus, bees do not compensate for low pollen quality by providing their progeny with larger pollen grains and/or higher quantities of nutritionally poor pollen.

Additionally, an interesting observation was the identification of *Solanum riparium* pollen in pot samples of *S. polysticta* and *S. depilis*. Although the amount calculated was very low (6.37 and 0.09, respectively), on the basis of its protein content *S. riparium* reach the position number 8 out of 24 plant species. In the same line, a recent study reported that pollen of *Solanum* genus had a higher protein concentration than most of plant genera visited by bees (Pamminger et al. 2019). Taking into account this evidence, we wonder why *S. riparium* was not more represented in quantity, nor collected by all bee species, because *Solanum* possess poricidal anthers, it requires pollination from specialized bees that use vibrations to release pollen (i.e., “buzz-pollination” bees) and, as far as we know, *Scaptotrigona* species do not perform buzz pollination. By the contrary, in sample plots of *M. rufiventris* we did not find *S. riparium* pollen, which is interestingly because other *Melipona* species perform buzz-pollination (Nunes-Silva et al. 2010, 2013) and one of them is an efficient pollinator of a *Solanum* species (Nunes-Silva et al. 2013). Probably the buzz-pollination in *Meliponini* species is more frequent than is currently known.

Other studies found that crude protein as the protein-to-lipid ratio in pollen are strongly correlated with plant phylogeny and pollinator dependency, as a consequence of selection imposed by pollinator preferences, leading pollen nutrient contents and ratios to co-evolved with the needs of their insect pollinator partners (Ruedenauer et al. 2019). It is also worth mentioning that a specific protein-to-lipid ratio has been observed to affect bumblebees, honeybees and *Osmia* bees foraging preferences, so it is necessary to deepen into this nutrient proportion in further studies with stingless bees (Vaudo et al. 2016; Vaudo et al. 2020).

The bipartite network of bees and plants showed a high degree of connectance, i.e. the number of observed interactions in relation to the total number of possible interactions. Likely, this is a consequence of the sampling method, as data from pollen pots reflect interactions that are not easy to observe in a plant-centered sampling. This seems to be the case, as we even recorded interactions with plants that were not detected in the sampling area of 160000 m² around each meliponary. Similar high connectance was obtained by Ferreira and Absy (2015), working with pollen data from

Melipona species in the Amazonian basin. Low modularity is also expected, given the small size of the network and the inclusion of similar bee species. Nestedness based on overlap and decreasing fills (NODF) has been reported robust to heterogeneous sampling (Rivera-Hutinel et al. 2012), the observed value indicates moderate to low nestedness, suggesting an intermediate level of hierarchical organization. Finally, specialization values may be driven by the overwhelming presence of *Capsicum* pollen in the pots. Further studies may clarify if this specialization, which seems to be associated to preferences for high quality pollen, remain stable or, more likely, change over the seasons in response to available floral resources.

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